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Uric Acid Is Closely Linked to Vascular Nitric Oxide Activity

Evidence for Mechanism of Association With Cardiovascular Disease

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OBJECTIVES The study was undertaken to determine whether the mechanism of association of elevated serum uric acid level (SUA) with cardiovascular disease (CVD) is secondary to a common link with vascular nitric oxide (NO) activity.

BACKGROUND Epidemiologic studies demonstrate an association of elevated SUA with CVD. The mechanism of this association is unknown, but both may be linked via an impairment in vascular NO activity. To examine this, we determined the relationship of SUA to vascular NO activity and to CVD risk. We then determined the effect of enhancing vascular NO activity on SUA.

METHODS In part 1, individuals with various degrees of CVD (n = 458) were surveyed and underwent measurement of flow-mediated brachial artery vasodilation (FMV), a measure of vascular NO activity. In part 2, we performed an analysis of data pooled from six separate clinical trials of a medical food designed to enhance vascular NO activity in individuals with CVD (n = 217 subjects representing 253 treatment periods) to determine the effect on SUA.

RESULTS In part 1, of all risk factors tested, SUA was second only to age in correlation with FMV, accounting for 7% ($p < 0.0001$) of the variability in FMV. Both SUA and FMV were related to the degree of disease risk ($p < 0.0001$ and $p = 0.00025$ by analysis of variance, respectively). By multivariate analysis, SUA did not continue to contribute significantly to the determination of FMV. In part 2, enhancement of FMV (5.8 ± 4 to 8.6 ± 5, $p < 0.0001$) was associated with a decrease in SUA (5.5 ± 1.5 to 5.0 ± 1.5, $p < 0.0001$). There was no placebo effect on FMV or SUA.

CONCLUSIONS These results suggest that the association of elevated SUA with CVD may be a consequence of an impairment of vascular NO activity. This may be owing to an ability of NO to modulate uric acid production through its influence on xanthine oxidase activity. (J Am Coll Cardiol 2001;38:1850–8) © 2001 by the American College of Cardiology

Many epidemiologic studies reported over the past 50 years have confirmed nearly consistently an association of elevated serum uric acid (SUA) level with cardiovascular disease (CVD), although not all have found that the correlation is independent of other risk factors (1–7). In addition to the issue of dependency of the association is the matter of the mechanism of the association. Several theories have been advanced, including those that implicate elevated SUA as a causative factor—for instance, by increasing platelet reactivity (8). Theories for elevated SUA as a consequence of vascular disease have also been considered. For example, a disturbance in renal urate handling appears to exist in patients with hypertension (9). Other researchers have proposed that elevated SUA is a consequence of a change in intracellular oxidative metabolism that occurs in diseased vessels (10). Still others have proposed that it is a manifestation of metabolic disease independent of the condition of the vasculature (11).

Intriguingly, almost all of the factors that correlate with SUA also diminish vascular nitric oxide (NO) activity. Elevated SUA is linked with obesity (3), dyslipidemia (12), hypertension (13), insulin resistance (14), male gender, aging, menopause (15,16), sedentary lifestyle (17), excessive alcohol intake and diuretic use (6,18,19). By various mechanisms, all of these factors have been shown to either reduce NO production and/or increase NO destruction (20).

Vascular NO activity is now known to affect enzymes involved in uric acid metabolism (21–23). It is perhaps through this common link by which all of these very different risk factors influence the level of SUA. Taken together with the role that vascular NO plays in the progression of CVD, a new plausible hypothesis for the mechanism of association of SUA with CVD emerges. Specifically, we hypothesized that endogenous NO activity may closely influence uric acid production. Should this be true, the following relationships should be found: 1) SUA should closely correlate with measures of vascular NO activity; 2) both SUA and measures of vascular NO should correlate with various cardiovascular risk factors; 3) vascular NO activity should relate inversely while SUA should relate directly to degree of CVD; and 4) modification of NO activity should affect SUA. Accordingly, we tested this hypothesis by investigating these relationships in a population with various degrees of CVD. We then examined the

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Abbreviations and Acronyms

ANOVA = analysis of variance
BMI = body mass index
CAD = coronary artery disease
CVD = cardiovascular disease
FMV = flow-mediated vasodilation
HDL = high-density lipoprotein
LDL = low-density lipoprotein
NO = nitric oxide
PAD = peripheral arterial disease
SUA = serum uric acid level

effect on SUA of an intervention designed to enhance vascular NO activity.

METHODS

Part 1: relationship of SUA to vascular NO. STUDY POPULATION. Participants were ambulatory adults in the San Francisco Bay area recruited in the years 1999 and 2000 through various media and who gave their informed consent. To assure a wide distribution of CVD risk factors, a portion of the recruitment was directed toward individuals with known cardiovascular risk factors or disease including specifically individuals with hypercholesterolemia, diabetes, angina and claudication. Spouses and relatives were encouraged to enroll either as those with or without risk factors. Information collected on participants included demographic data and a medical, smoking and physical activity history. Participants underwent height, weight, supine resting blood pressure and fasting serum laboratory measurements as well as a determination of in vivo vascular NO activity. The physical activity survey used was the well-validated seven-day physical activity recall (24).

Laboratory analysis included measures of serum total, calculated low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low density lipoprotein cholesterol, triglycerides, homocysteine, Lp(a), insulin, glucose, creatinine, uric acid and fibrinogen (by Unilab, San Jose, California, or by SmithKline Beecham Laboratories, Van Nuys, California). Participants taking allopurinol were excluded from analysis. The data collected allowed for the stratification of the population into four cardiovascular risk levels. The first group consisted of those who were free of any identifiable disease or risk factors for CVD determined by review of medical records, history, physical examination and laboratory measurements and as defined below; the second group included those with one or more risk factors but no identifiable disease; the third group included those who had been independently diagnosed by traditional methods as having atherosclerotic CAD, cerebral arterial disease or peripheral arterial disease (PAD) including those who were symptomatic but were free of any cardiovascular events; the fourth group included those with a history of myocardial infarction or stroke. Risk factors excluded age and male gender and included pack-years of tobacco use, presence of diabetes, family history of premature coronary artery disease (CAD), elevated serum cholesterol with total cholesterol >230 mg/dl or LDL >160 mg/dl, obesity with body mass index (BMI) >32 kg/m^2, sedentary lifestyle with physical activity score <18, hypertension with supine systolic blood pressure >160 mm Hg or diastolic blood pressure >95 mm Hg, or the diagnosis of CAD, PAD or a history of a cardiovascular event (either myocardial infarction or stroke).

VASCULAR NO ACTIVITY. The NO-mediated vascular function was measured while subjects were fasting by assessing brachial artery flow-mediated vasodilation (FMV) using high-resolution ultrasound and software (15-MHz vascular probe, Acuson Sequoia, Mountain View, California; Brachial Tools, Medical Imaging Applications, Iowa City, Iowa). The FMV of the human brachial artery has been shown to be almost exclusively due to NO release by the endothelium because inhibitors of NO synthase completely abolish FMV (25). The method used is described previously in detail (26). Height-adjusted values of FMV are expressed in terms of percent increase from baseline measures.

Statistical analysis. Dichotomous variables were converted to dummy variables, and all variables were assessed for normality. Those variables found to have a skewed distribution were log-transformed. All variables or their log-transforms were converted to Z-scores so that relative strengths of relationships could be assessed. Univariate relationships were evaluated with Pearson's correlation coefficients. To determine which variables would be most predictive of FMV in a multivariable setting, manual reverse, stepwise selection procedures were employed to identify the most parsimonious model. Variables were selected for removal based on Pearson coefficients sequentially until an adjustment in the F statistic became significant. To assess the relationship of FMV and SUA to cardiovascular risk levels, analysis of variance (ANOVA) was used to compare variables between populations. The Fisher least significant difference was used as a post hoc test. Data in text and tables are presented as mean ± SD, whereas data in graphs are presented as mean ± SEM.

Part 2: effect of enhancing vascular NO on SUA. STUDY DESIGN. The second part of this study was an analysis of pooled data from subjects (n = 217 unique subjects representing 253 treatment periods) participating in any of six separate randomized, double-blind, placebo-controlled studies of a medical food bar designed to increase vascular NO activity. Subjects included in this analysis were adults with either symptomatic CAD (n = 36; crossover design on two weeks' treatment [27], and n = 10; on one-week treatment, unpublished data), symptomatic PAD (n = 41; on two weeks' treatment [28] and n = 46; on 12 weeks' treatment, unpublished data) or with hypercholesterolemia (screening total cholesterol >230 mg/dl and LDL cholesterol >160 mg/dl, n = 43; on one-week treatment and n = 41; on two weeks' treatment [26]) and who gave their

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Table 1. Pearson Correlations of Cardiovascular Disease Risk Factors and Other Markers With Serum Uric Acid Level

	Full Population (n = 458)		Men (n = 211)		Women (n = 247)	
Variable	Coefficient	p Value	Coefficient	p Value	Coefficient	p Value
Creatinine	0.486	<0.0001	0.235	<0.0001	0.381	<0.0001
Gender (dv)	0.440	<0.0001	—	—	—	—
Insulin*	0.370	<0.0001	0.400	<0.0001	0.354	<0.0001
HDL cholesterol	−0.359	<0.0001	−0.330	<0.0001	−0.148	0.03
Homocysteine*	0.316	<0.0001	0.078	0.3	0.404	<0.0001
BMI*	0.313	<0.0001	0.344	<0.0001	0.310	<0.0001
Triglycerides*	0.296	<0.0001	0.412	<0.0001	0.260	0.0001
Height	0.277	<0.0001	−0.070	0.4	−0.097	0.2
Diuretic use (dv)	0.269	<0.0001	0.236	0.002	0.347	<0.0001
Fasting glucose*	0.254	<0.0001	0.183	0.02	0.273	<0.0001
Antihypertensive use (dv)	0.216	<0.0001	0.147	0.06	0.204	0.003
Mean blood pressure	0.184	0.0003	0.052	0.5	0.263	<0.0001
Age	0.138	0.006	−0.013	0.9	0.292	<0.0001
Fibrinogen	0.131	0.01	0.251	0.001	0.022	0.08
Pack-years cigarettes*	0.085	0.09	0.059	0.4	0.016	0.8
LDL cholesterol*	0.044	0.4	0.124	0.1	0.079	0.2
EtOH consumption*	0.038	0.5	−0.056	0.5	−0.032	0.6
Lp(a)*	−0.034	0.5	−0.011	0.9	−0.062	0.4
Family history of premature CAD (dv)	−0.019	0.7	0.072	0.3	−0.083	0.2
Total cholesterol*	−0.016	0.6	0.155	0.04	0.093	0.2
Physical activity*	−0.012	0.8	−0.161	0.04	0.023	0.7

*Values have been log-transformed.

BMI = body mass index; CAD = coronary artery disease; dv = converted to dummy variables; EtOH consumption = ethanol drink (standardized) per day; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

informed consent. Upon meeting eligibility criteria, subjects underwent laboratory analysis for measures of basic serum chemistries including cholesterol and SUA. A subpopulation consisting of those subjects who participated in the crossover angina study and one of the hypercholesterolemia studies (n = 56 and 55 active and placebo treatment periods, respectively) also underwent a vascular function study as described above. Subjects in all studies were then randomized to receive either two active bars or two placebo bars per day for 1, 2 or 12 weeks followed by repeat laboratory and vascular function studies. Subjects in all these studies signed informed consent, and all procedures followed were in accordance with the ethical standards of the approving independent review board and with the Helsinki Declaration of 1975, as revised in 1983.

Study product. The active bar is an L-arginine-enriched nutrient bar (Cooke Pharma, Belmont, California) that was designed to enhance vascular NO activity. Each 50-g active bar contains 3.3 g of L-arginine as well as antioxidant vitamins and folic acid in a soy protein and oat fiber base. This bar has been described previously and has been shown in previous studies to enhance brachial artery FMV in subjects with hypercholesterolemia, claudication and stable angina (26–28). Placebo bars are similar to the active bar with respect to weight, flavor, caloric, carbohydrate, protein, fiber and fat content but is devoid of added L-arginine and vitamins.

Statistical analysis. Categorical demographic data was compared among treatment groups using chi-square analysis. The ANOVA was used to compare values from treatment groups with a control group. The effect of treatments on FMV, SUA and other laboratory measures was compared by univariate ANOVA controlling for baseline values.

RESULTS

Part 1: relationship of SUA to FMV. Four hundred fifty-eight participants (211 men, 247 women, mean age 60 ± 14 years [SD], range 25 to 88 years) enrolled in the study. Seventy-nine participants had no identifiable risk factors (as defined in the Methods section) or disease. The remainder had one or more risk factors and/or were diagnosed with CVD: active tobacco use (n = 32), diabetes (n = 43), hypercholesterolemia (n = 150), obesity (n = 67), sedentary lifestyle (n = 96), hypertension (n = 20), a family history of premature CAD (n = 118), with a diagnosis of CAD (n = 83), stable angina (n = 61), intermittent claudication (n = 24), and with a history of a cardiovascular event (n = 28).

The mean SUA level for the population was 4.8 ± 1.5 mg/dl (range 1.7 to 10.7 mg/dl). Men had a significantly higher SUA than women (5.5 ± 1.3 mg/dl vs. 4.2 ± 1.4 mg/dl, $p < 0.0001$). The SUA demonstrated univariate multicollinearity with several physical and biochemical factors (Table 1). In addition to height, serum creatinine and diuretic and antihypertensive use, SUA correlated significantly with several markers of CVD risk: gender, age, BMI, mean blood pressure, fasting serum insulin, HDL choles-

Table 2. Pearson Correlations of Cardiovascular Disease Risk Factors and Other Markers With Flow-Mediated Vasodilation

	Full Population (n = 458)		Male (n = 211)		Female (n = 247)	
Variable	**Coefficient**	**p Value**	**Coefficient**	**p Value**	**Coefficient**	**p Value**
Age	−0.305	<0.0001	−0.358	<0.0001	−0.264	<0.0001
Uric acid	−0.258	<0.0001	−0.250	0.001	−0.175	0.01
Creatinine*	−0.254	<0.0001	−0.251	0.001	−0.208	0.002
Mean blood pressure	−0.215	<0.0001	−0.248	0.0003	−0.165	0.009
Homocysteine*	−0.211	<0.0001	−0.234	0.0007	−0.028	0.7
Insulin*	−0.181	0.0001	−0.263	0.0001	−0.049	0.4
HDL cholesterol	0.169	0.0003	0.134	0.05	0.083	0.2
Gender (dv)	−0.150	0.001	—	—	—	—
Fasting glucose*	−0.153	0.003	−0.114	0.1	−0.167	0.01
Physical activity	0.134	0.004	−0.214	0.002	0.073	0.3
Triglyceride*	−0.133	0.005	−0.164	0.02	−0.103	0.1
Pack-years cigarette use*	−0.117	0.01	−0.179	0.009	0.001	0.99
Fibrinogen	−0.109	0.02	−0.164	0.02	−0.068	0.3
Antihypertensive use (dv)	−0.108	0.02	−0.165	0.02	−0.009	0.9
Diuretic use (dv)	−0.102	0.03	−0.045	0.5	−0.153	0.02
BMI*	−0.100	0.03	−0.158	0.02	−0.041	0.5
Family history of premature CAD (dv)	−0.093	0.05	0.030	0.7	−0.196	0.002
LDL cholesterol*	−0.080	0.09	−0.137	0.05	−0.045	0.5
EtOH consumption*	−0.048	0.3	0.068	0.3	−0.127	0.05
Lp(a)*	−0.044	0.4	−0.049	0.5	−0.031	0.6
Total cholesterol*	−0.039	0.4	−0.141	0.04	−0.025	0.7
Height	−0.032	0.5	0.126	0.07	0.107	0.09
HRT (dv)	—	—	—	—	−0.027	0.7

*Values have been log-transformed.
HRT = estrogen hormone replacement therapy; other abbreviations as in Table 1.

terol, homocysteine, triglycerides, glucose and fibrinogen. In addition to these factors, SUA also correlated with physical activity in men. Thus, there was substantial collinearity between SUA and factors that might affect FMV.

Mean FMV for the entire population was 6.7 ± 3.8% (range −0.8 to 25%). With the use of height correction of vessel diameter, there was no correlation of FMV to subject height. As with SUA, FMV also demonstrated multicollinearity with several physical and biochemical markers (Table 2, Fig. 1). The strongest correlation was with participant age followed by SUA and mean blood pressure. Indeed, 9%, 7% and 5% of the variability in FMV was attributed respectively, to age, SUA and mean blood pressure ($p < 0.0001$ for each). Also, FMV correlated with physical activity, gender, BMI, family history of premature CAD, pack-years of tobacco use and diuretic and antihypertensive use, as well as serum levels of homocysteine, insulin, HDL cholesterol, fasting glucose and fibrinogen. Nearly all these factors were shown to be significant in men, whereas only a few of them were found to be significant in women. In addition, men demonstrated a correlation with total cholesterol, while women manifested a correlation with alcohol consumption.

The observed multicollinearity of both SUA and FMV with the physical and CVD risk factors is an anticipated outcome of this hypothesis. Another anticipated outcome is that, despite SUA having a close correlation with FMV, it should ultimately be found not to be an independent determinant of FMV given that enough relevant factors are represented in a multivariate model. Therefore, reverse, stepwise multivariate regression beginning with all measured variables was performed on the entire population as well as on the male and female population separately. For the entire population, removal of SUA from the model affected the overall accounted variability (R^2 of the model) very little as long as ≥10 of the most significant variables remained in the model. With 13 variables in the model, 25% of all variability in FMV is accounted. The factors that remain significant determinants of FMV are age ($R = -0.241$, $p < 0.0001$), creatinine ($R = -0.166$, $p = 0.003$), triglycerides ($R = -0.120$, $p < 0.04$), LDL/HDL ratio ($R = -0.129$, $p < 0.04$) and mean blood pressure ($R = -0.096$, $p = 0.05$). For the population of men, SUA continued to remain a significant factor with 10 variables present (32% of variability in FMV accounted for). Only age ($R = -0.343$, $p < 0.0001$) and SUA ($R = -0.157$, $p = 0.05$) were significant contributors. For the population of women, the factors that remained significant included age ($R = -0.203$, $p < 0.005$), family history of premature CAD ($R = -0.163$, $p = 0.01$) and serum triglyceride level ($R = -0.142$, $p = 0.05$).

Another anticipated outcome of this hypothesis is that SUA should relate directly, and measures of vascular NO should relate inversely, with degree of CVD. To examine this, we stratified the population into four CVD risk levels as defined in the Methods section (Table 3). By this

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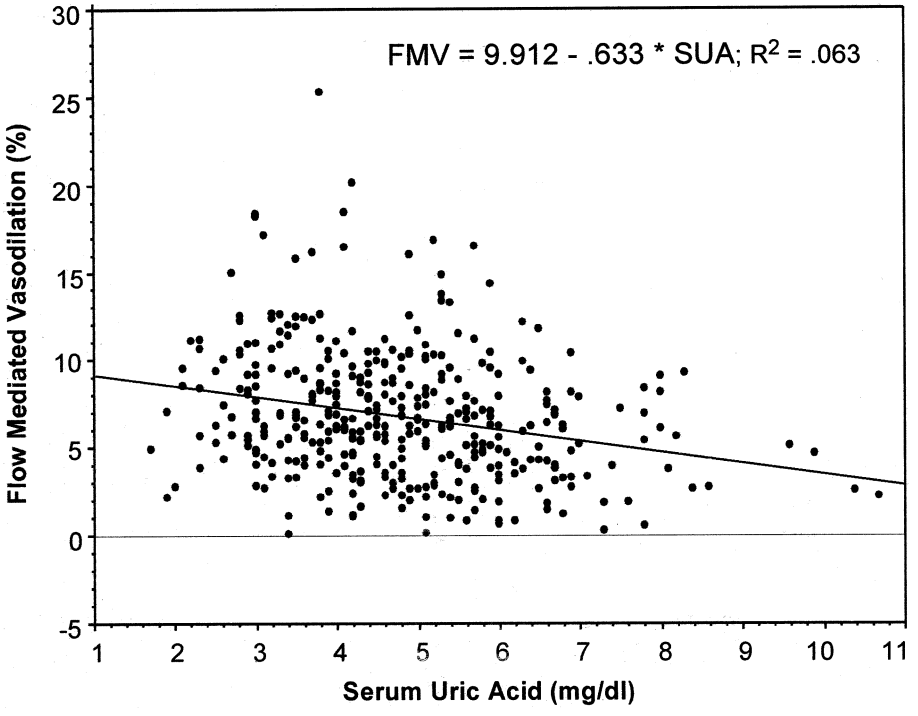

Figure 1. Relationship of serum uric acid level (SUA) to flow-mediated vasodilation (FMV) for a population of men and women (n = 458) with and without cardiovascular disease risk factors.

stratification, there was a stepwise increase in SUA ($p = 0.0001$ by ANOVA) and a stepwise decrease in FMV ($p < 0.0001$) with increasing degree of CVD. Indeed, a plot of the mean SUA versus mean FMV for these groups form a line with an R^2 of 0.995 (Fig. 2).

Part 2: effect of the L-arginine-enriched medical food. To assess the effect of altering vascular NO activity on SUA, we performed an analysis of pooled data from clinical studies of the medical food bar. The demographics and medical data of the population that made up this analysis are

Table 3. Demographic, Physical and Biochemical Data of Subjects in Part 1 Separated by Risk Level

Variable	No Risk	At Risk	CVD Diagnosis	CV Event
N	75	299	8	16
Age (yrs)	54 ± 16	60 ± 13‡	67 ± 10‡	69 ± 10‡
Male (%)	41	40	70†	81†
Caucasian (%)	86	87	100	85
Diabetic (%)	0	10	18†	0
Current smoker (%)	0	9*	3	0
Tobacco use (pack-years)	6 ± 17	10 ± 19	22 ± 30‡	17 ± 18
BMI (kg/m^2)	24 ± 3	27 ± 6‡	27 ± 5†	26 ± 3
Mean blood pressure (mm Hg)	88 ± 8	92 ± 10†	95 ± 9‡	94 ± 9*
Physical activity score	77 ± 52	64 ± 63	51 ± 52*	37 ± 42*
EtOH (drinks/day)	4 ± 7	3 ± 6	4 ± 6	6 ± 8
Family history of CAD (%)	0	31‡	31‡	25*
Diuretic use (%)	5	9	7	13
Antihypertensive use (%)	2	20	47‡	56‡
Total cholesterol (mg/dl)	193 ± 27	224 ± 43‡	200 ± 43	176 ± 37
LDL cholesterol (mg/dl)	112 ± 24	137 ± 39‡	121 ± 39	103 ± 40
Triglycerides (mg/dl)	104 ± 46	145 ± 99†	154 ± 91†	105 ± 43
Fasting glucose (mg/dl)	89 ± 11	101 ± 33†	106 ± 20†	96 ± 13
Insulin (mcU/ml)	9 ± 4	11 ± 7*	14 ± 8‡	12 ± 7
Homocysteine (mg/dl)	9 ± 2	9 ± 3	11 ± 5‡	13 ± 9‡
Lp(a) (mg/dl)	16 ± 10	35 ± 35‡	42 ± 44‡	32 ± 30
Fibrinogen (mg/dl)	302 ± 71	321 ± 78	336 ± 104*	360 ± 98*
Creatinine (mg/dl)	0.9 ± 0.1	0.9 ± 0.2	1.1 ± 0.2†	1.5 ± 0.9‡

Values are mean ± SD where applicable. *$p < 0.05$; †$p < 0.005$; ‡$p < 0.0001$ by analysis of variance.
CV = cardiovascular; CVD = cardiovascular disease; other abbreviations as in Table 1.

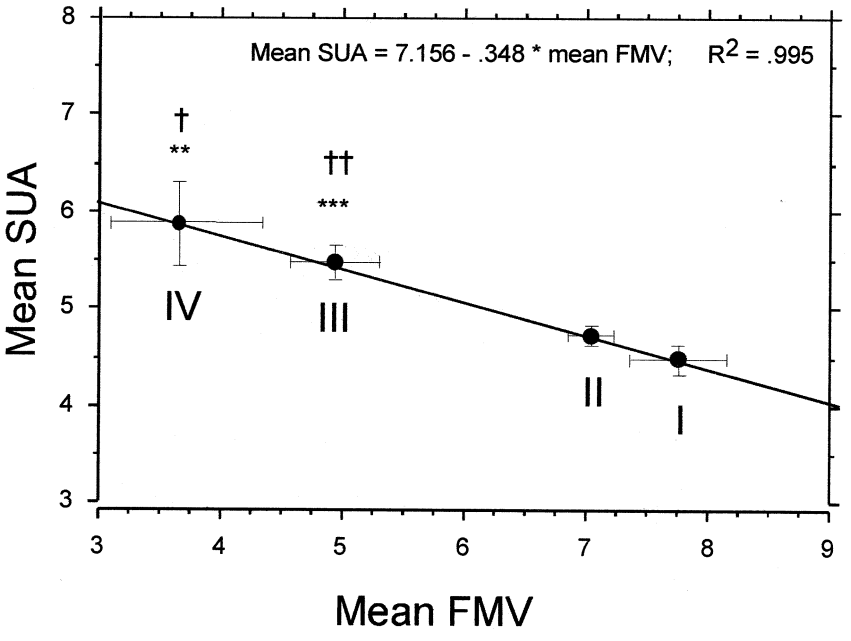

Figure 2. Relationship of flow-mediated vasodilation (FMV) and serum uric acid level (SUA) with degree of cardiovascular disease (CVD) risk. There is a stepwise decrease in FMV and a stepwise increase in SUA with increasing risk. Regression plot of mean values for each risk group forms is nearly linear. The four risk groups are: **I** = those with no disease or risk factors for cardiovascular disease; **II** = those with one or more risk factors but no CVD identified by history, physical examination, laboratory analysis or review of medical records; **III** = those with a diagnosis of CVD but without events; **IV** = those with a history of events including myocardial infarction and stroke. Values are mean ± SEM. **$p < 0.01$; ***$p < 0.001$ for FMV and †$p < 0.05$; ††$p < 0.01$ for SUA.

shown in Table 4. The FMV was equally reduced in both the active and placebo groups at baseline compared to healthy controls, who were free of risk factors and who were selected from a separate database and matched for age and gender (8.6 ± 4.9%, n = 14). Treatment with the active bar (n = 56), significantly increased FMV while treatment with placebo (n = 55) had no effect on FMV ($p < 0.0001$, Fig. 3A). In parallel to the effects of the active bar on FMV, active treatment resulted in a significant reduction in uric acid, whereas treatment with placebo had no effect ($p < 0.0001$ Fig. 3B). Neither active treatment nor placebo treatments affected BMI or measures of serum total, HDL or LDL cholesterol, fasting glucose, insulin or creatinine, suggesting that changes in NO-independent determinants of SUA were not responsible for these observations.

DISCUSSION

The important findings in this study are:

Table 4. Baseline Demographic and Medical Data of Subjects in Part 2 (n = 217 unique)

Variable	Active	Placebo	p Value
Age (mean ± SD)	63 ± 11	65 ± 10	0.3
Men (%)	66	69	0.6
Caucasian (%)	93	95	0.7
Current use of tobacco (%)	10	17	0.1
Diagnosis of diabetes (%)	9	4	0.2
History of hypercholesterolemia (%)	70	62	0.2
History of hypertension (%)	43	31	0.2

1) SUA correlates closely with the measure of in vivo vascular NO activity, FMV;
2) both SUA and FMV correlate with CVD risk factors;
3) both SUA and FMV are significantly associated with the degree of cardiovascular risk; and
4) alteration of NO activity, as measured by FMV, is associated with an alteration in SUA.

Relationship of SUA with FMV and other risk factors. Consistent with the hypothesis presented, SUA correlates closely with a measure of vascular NO activity. Indeed, only age correlates more closely with FMV, suggesting a close link between vascular NO activity and SUA. Despite the relatively close univariate correlation, this hypothesis would predict that SUA should ultimately drop out of the list of factors independently determining FMV if sufficient sample size, as well as the number and choice of variables, is represented. Using multivariate analysis and originally including 22 measured variables, SUA was removed along with 9 other variables before a significant impact on our model occurred, although SUA continued to be a significant predictor in the population of men.

Another anticipated consequence of the theory is that both SUA and FMV correlate with measures of the many risk factors of CVD. Our observations fit with this theory and, in general, with what is reported in the literature; there is a stronger relationship of both SUA and FMV in the male gender; both SUA and FMV are related to age, blood pressure, gender, BMI, serum levels of homocysteine, insulin, HDL, fasting glucose, triglycerides as well as diuretic

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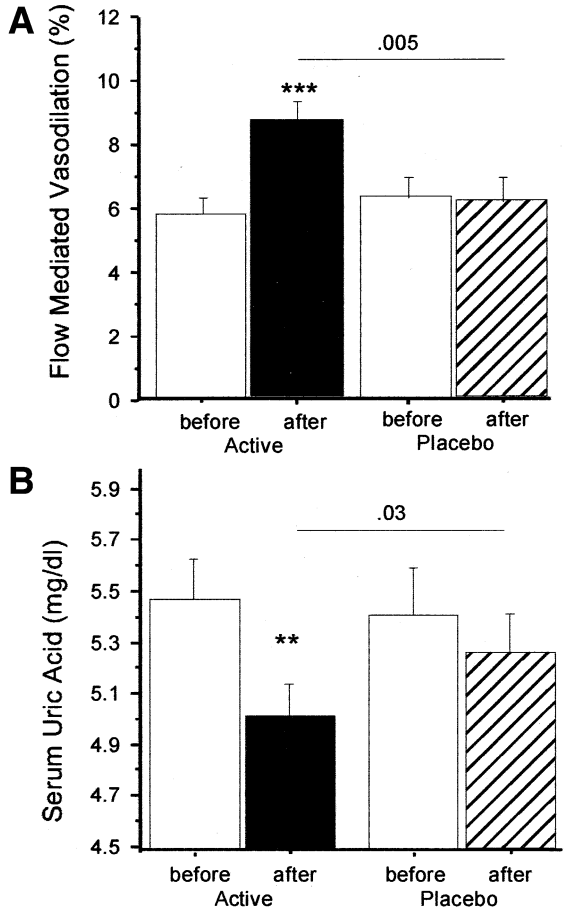

Figure 3. Effect of L-arginine-enriched medical food on flow-mediated vasodilation (FMV) and serum uric acid level (SUA). **(A)** FMV (n = 111) and **(B)** SUA (n = 253) before and after intervention designed to enhance vascular NO production. Values are mean ± SEM. $^{**}p < 0.01$; $^{***}p < 0.0001$ for overall model by univariate analysis of variance controlled for baseline measures; p values for treatment interaction at time after are given above bars.

and antihypertensive use. All these factors would be expected to affect vascular NO activity, which would, in turn, affect SUA. These are findings previously reported for SUA (19,29). However, contrary to what has been reported (7,30), we did not observe an effect of estrogen hormone replacement therapy on SUA or FMV in women even when correcting for any skew in age between users and nonusers.

SUA, FMV and degree of CVD risk. Consistent with the current hypothesis and with large epidemiologic studies, our study supports the association of SUA with degree of CVD. We also demonstrate that FMV is inversely associated with the level of cardiovascular risk. Previous studies investigating the relationship of brachial artery FMV with CVD or risk factors have been limited to the association of impairment of brachial artery FMV with presence or absence of disease. The relationship we observed here is not surprising given that vascular NO activity appears to be increasingly impaired with disease progression. Indeed, it is well established that vascular NO activity is diminished in the presence of risk factors long before there is any evidence of overt disease (31). A most intriguing finding emerges in this study from these relationships; there is a strong linear correlation between the mean SUA levels and mean FMV for the four groups with virtually no overlap of SDs and an $R^2 = 0.995$. This finding highlights the clinical significance of the relationship of SUA and FMV with degree of cardiovascular risk.

Effect of intervention. The final observation from this study that is consistent with the current hypothesis is the effect of manipulating vascular NO activity on SUA. Treatment with the active therapy increased FMV without affecting other factors known to influence SUA levels. Because medical food is enriched with nutrients known to enhance vascular NO activity and because the intervention did not affect other factors known to influence SUA, it is reasonable to suggest that the treatment acted to reduce SUA by an NO-dependent mechanism. Further evidence to suggest that the mode of action of the intervention was via enhancement of vascular NO activity is the effect of therapy on symptoms during exertion. Patients with symptoms of angina or claudication were capable of increasing symptom-limited exercise endurance without affecting the degree of ischemia (27,28). This would suggest that the mechanistic link with uric acid would then be via an action downstream to vascular NO activity.

Potential mechanisms of association. Nitric oxide is known to interact with both the flavin prosthetic site and the molybdenum ion at the active site of xanthine oxidase, thereby interfering with its activity or producing a desulfo-type inactive enzyme (22). Therefore, as vascular NO activity diminishes, its suppression of uric acid production is lifted. Indeed, Cote et al. (23) demonstrated that L-arginine administration resulted in a decrease in xanthine oxidase activity in hypoxic rats. Peroxynitrite anion may also be involved in the mechanism of reduction of uric acid levels. The activity of peroxynitrite anion, which forms from the reaction of NO with superoxide anion, may increase in parallel with the activity of NO. Uric acid is quickly oxidized by peroxynitrite, thereby removing both (21). Another potential mechanism that is also consistent with the data of Tykarski (9) is that enhanced FMV leads to improved renal blood flow and enhanced excretion of uric acid.

In addition to providing a link for how the multitude of very different risk factors affect SUA levels, this proposed mechanism explains the finding of other investigators that support other proposed mechanisms. The relationship of vascular NO activity to renal clearance of urate mentioned above is one example. As another example, Newland (32) presented data supporting an increase in platelet reactivity associated with elevated SUA, implicating a causal relationship of elevated SUA in CVD. However, diminished vascular NO activity results in enhanced platelet reactivity through mechanisms independent of uric acid. Thus, a reduction in NO activity leads to both an increase in platelet activity and, independently, to elevated SUA.

Leyva et al. (10) proposed that the increase in CVD was secondary to an effect of uric acid to decrease oxidative metabolism after finding a good inverse correlation between SUA and maximal oxygen uptake in heart failure patients. However, heart failure patients are known to have reduced vascular NO activity, and by enhancing vascular NO activity with L-arginine administration, exercise capacity in heart failure patients is improved (33). Here again, a reduction in NO activity in heart failure leads to a reduction in exercise capacity and, independently, an elevated SUA.

This effect of vascular NO on SUA may provide a regulatory control mechanism of oxidative stress. Because uric acid is an antioxidant with the ability to remove peroxynitrite and slow lipid peroxidation, Ames and Hochstein have proposed that it may be an evolutionary substitute to compensate for the loss of ability by higher primates to synthesize ascorbic acid (34,35). In the case where the endothelium is healthy and oxidative stress is low, NO activity would be expected to be sufficient to put a brake on xanthine oxidase activity restricting the production of uric acid. In the presence of risk factors, however, oxidative stress increases, vascular NO activity wanes, and the brake on xanthine oxidase activity is removed. The subsequent enhanced uric acid production then helps to restore oxidative stress toward normal. This is consistent with data from Nieto et al. (36), who demonstrated that individuals with atherosclerosis had higher serum antioxidant capacity than matched controls. The difference was almost entirely explained by increased SUA (36). This regulatory control mechanism is effective as long as xanthine oxidase does not generate superoxide anion. This process may occur in the presence of hypoxia when the enzyme transfers electrons to molecular oxygen rather than to NAD^+ during the formation of uric acid (37). Under this condition, xanthine oxidase activity contributes to, rather than reduces, oxidative stress.

Conclusions. The data from this study confirms the relationship of SUA to CVD. Furthermore, the relationship of SUA to vascular NO activity and to risk factors for CVD provides evidence that the association of SUA with CVD may be secondary to diminished vascular NO activity possibly through disinhibition of xanthine oxidase.

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